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Pluteus variabilicolor and *Volvopluteus earlei*, new records for Pakistan

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Abstract—*Pluteus variabilicolor* and *Volvopluteus earlei* are reported as new records for the Pakistani funga, with the former representing a new record for South Asia and the latter, a second report from Asia. Morphological descriptions are accompanied by colored photographs of basidiomata and microscopic structures. Identity of both species is confirmed by ITS sequence analyses. Comparison with previous descriptions and data on their distributions are briefly discussed.

Key words—*Agaricales*, phylogeny, *Pluteaceae*, *P.* sect. *Hispidoderma*, taxonomy

Introduction

Pluteaceae Kotl. & Pouzar (*Agaricales*) is a family of saprophytic fungi widely distributed in major parts of the world (Kirk & al. 2008). Molecular reassessment of the family (Justo & al. 2011a) supports two genera: *Pluteus* Fr. and *Volvopluteus* Vizzini & al.

Pluteus, the larger genus, comprises >300 species (Kirk & al. 2008, He & al. 2019), distributed worldwide especially in boreal to tropical forested regions (Menolli & al. 2014). *Pluteus* species are primarily lignicolous and grow on partially or well-rotted angiosperm and gymnosperm wood (Orton 1986, Singer 1986, Vellinga 1990). The presence of free lamellae, pinkish

spore-print, inverse hymenophoral trama, and inamyloid basidiospores and lack of universal veil are some key *Pluteus* characters (Vellinga & Schreurs 1985, Orton 1986, Singer 1986, Vellinga 1990, Heilmann-Clausen 2012). Only eight *Pluteus* species have been reported from Pakistan: *P. ephebeus* (Fr.) Gillet (as *P. murinus* Bres.), *P. escharites* (Berk. & Broome) Sacc., *P. fusconigricans* (Berk. & Broome) Sacc., *P. leoninus* (Schaeff.) P. Kumm., *P. palumbinus* (Berk.) Sacc., *P. pellitus* (Pers.) P. Kumm., *P. petasatus* (Fr.) Gillet, and *P. pulverulentus* Murrill (Ahmad 1980, Iqbal & Khalid 1996, Ahmad & al. 1997).

Volvopluteus, typified by *V. gloiocephalus* (DC.) Vizzini & al., was erected to accommodate some species formerly placed in *Volvariella* (Justo & al. 2011a). The genus is distinguished from *Pluteus* and *Volvariella* by a gelatinous pileipellis that is an ixocutis with relatively narrow hyphae (average ≤15 µm diam.) and average basidiospore length >11 µm (Justo & al. 2011a). Only one *Volvopluteus* species, *V. gloiocephalus*, has been reported from Pakistan (Ahmad 1980 and Iqbal & Khalid 1996, as *Volvariella speciosa* (Fr.) Singer).

This paper, part of a larger project dealing with the exploration of macrofungi in northern Khyber Pakhtunkhwa reports two pluteaceous species as new records for the funga of Pakistan: *Pluteus variabilicolor* and *Volvopluteus earlei*.

Materials and methods

Collection & morphological Studies

Specimens were collected during macrofungal surveys conducted between 2014 and 2019 in different parts of Pakistan. The specimens of *P. variabilicolor* were collected in village Ingaro Dherai, district Swat, Khyber Pakhtunkhwa province. Those of *V. earlei* were collected in the same locality and in the Sheikupura and Muzaffargarh districts of Punjab province. Basidiomata were dug out to the extreme base (to obtain some wood with specimens of *Pluteus* and volva with those of *Volvopluteus*) with a sharp knife. The specimens were photographed in their natural habitat and important macro-morphological characters were noted when fresh. Colors were coded according to soil color chart of Munsell (1975). Terminology for macroscopic and microscopic features follows Vellinga (1988). The specimens were dried in sun and deposited in the herbarium of the University of Swat, Kanju Township, Pakistan (SWAT) and the herbarium of the University of the Punjab, Lahore, Pakistan (LAH).

Microscopic descriptions are based on free-hand sections from dried specimens mounted both in distilled water and in aqueous potassium hydroxide solution (5% w/v), with aqueous Congo red added for contrast. Basidiospore measurements are based on 60 elements randomly selected from all the available basidiomata;

x presents the mean length and width of the basidiospores. The abbreviation Q refers to the length : width (L/W) ratio, with Q_m = the average length : width ratio.

DNA extraction, PCR amplification, sequencing

Genomic DNA from dried herbarium specimens was extracted using modified CTAB method of Gardes & Bruns (1993). The internal transcribed spacer region (ITS = ITS1-5.8S-ITS2) was amplified using the primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990). Polymerase chain reactions (PCR) were performed in 25 µL volume aliquots. Amplification involved initial 4 minutes denaturation at 94 °C, 40 cycles of 1 minute at 94 °C, 1 min at 55 °C, 1 min at 72 °C, and a final extension of 10 minutes at 72 °C (Naseer & al. 2020). PCR products were visualized in a UV illuminator loaded in 1% agarose gel added with 3 µL ethidium bromide. Purification and sequencing of the PCR amplicons were carried out at Beijing Genomic Institute (BGI) Hong Kong.

Sequence alignment & phylogenetic analysis

The forward and reverse reads were assembled into a consensus sequence using BioEdit software v. 7.2.5 (Hall 1999). The consensus sequences were used for a GenBank search, using the NCBI Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Matching sequences, especially those published in Justo & al. (2011b) and Lezzi & al. (2014), were downloaded for further phylogenetic analysis. Separate datasets were used for *Pluteus* and *Volvopluteus*.

Multiple sequences were aligned using online Multiple Alignment using Fast Fourier Transform (MAFFT) algorithm at European Bioinformatics Institute website (https://www.ebi.ac.uk/Tools/msa/mafft/). The phylogenies were inferred by maximum likelihood (ML) analysis using model selection for the best DNA analysis in MEGA6 software (Tamura & al. 2013). Jukes-Cantor model Models with the lowest BIC scores (Bayesian Information Criterion) was considered to describe the substitution pattern the best. Non-uniformity of evolutionary rates among sites were modeled using a discrete gamma distribution (+G) with 5 rate categories and assuming that a certain fraction of sites is evolutionarily invariable (+I). The phylogenetic analyses included 1000 bootstrap replicates.

Phylogenetic results

The final *Pluteus* ITS dataset comprised 37 sequences representing *P.* sect. *Hispidoderma* Fayod including the two sequences from our new Pakistani specimens. *Pluteus diettrichii* Bres. (HM562143) of *P.* sect. *Celluloderma* Fayod was used as outgroup following Lezzi & al. (2014). Maximum likelihood analysis convincingly clustered the Pakistani collections with other sequences of *P. variabilicolor* in the *Leoninus* clade of *P.* sect. *Hispidoderma* with a 100% bootstrap value (Fig. 1).

The ITS consensus sequences from our three new Pakistani *Volvopluteus* collections matched 99–99.5% with multiple *V. earlei* sequences (HM562205,

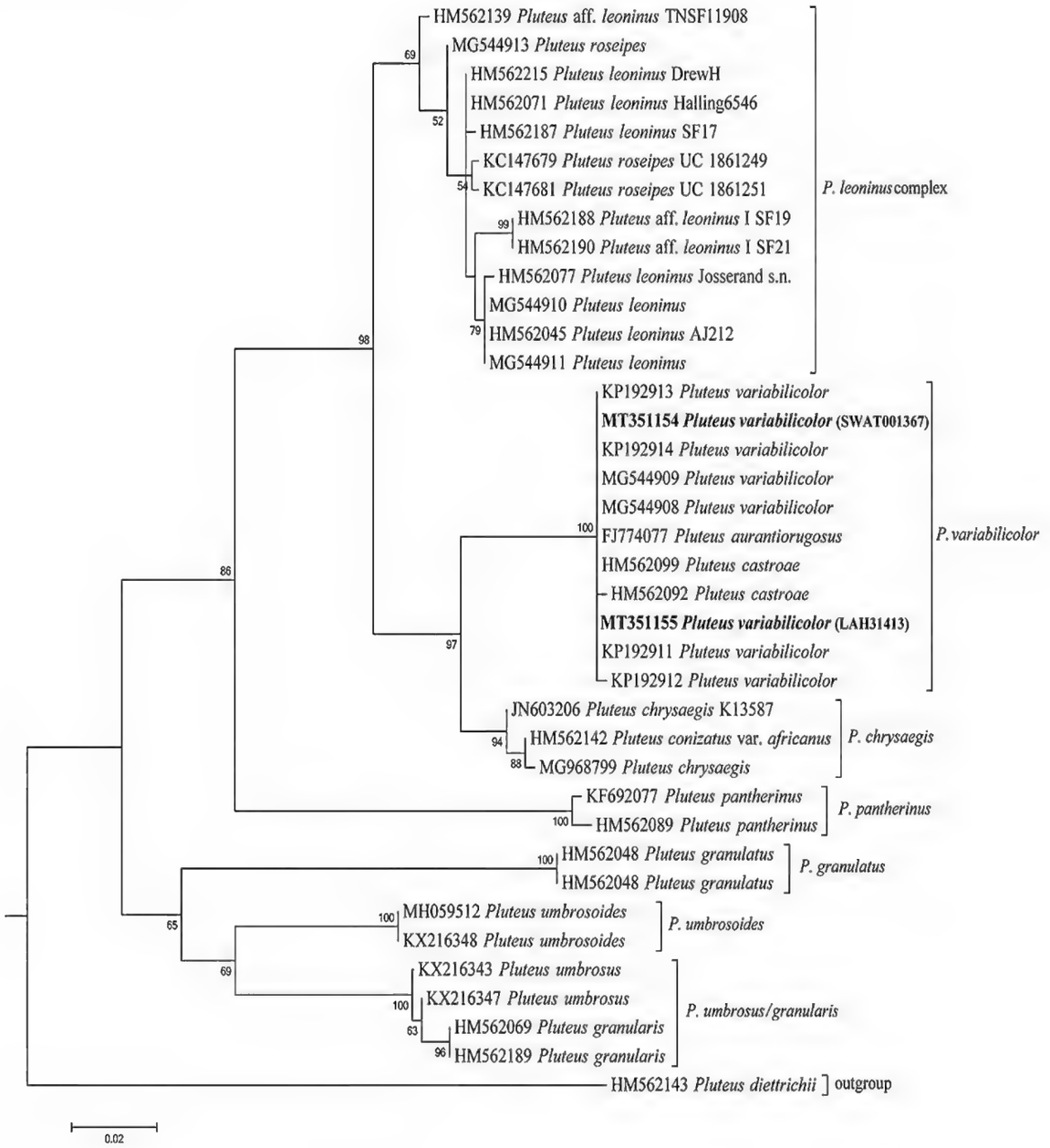

Fig. 1: Maximum likelihood phylogram of *Pluteus variabilicolor* and related species inferred from ITS data. Bootstrap values ≥50% are presented above nodes. Newly generated sequences of *P. variabilicolor* are presented in bold.

HM562205, HM246496, HM246497, HM246498, HM246499, MK204987, MK204989) in GenBank. The final *Volvopluteus* dataset comprises 19 sequences including the three sequences from the new Pakistani specimens and from *Pluteus heteromarginatus* Justo (HM562058) and *P. longistriatus* (Peck) Peck. (HM562082), the outgroup cited in Justo & al. (2011b). Sequences of the Pakistani collections clustered with other sequences of *V. earlei*, confirming their identity (Fig. 2).

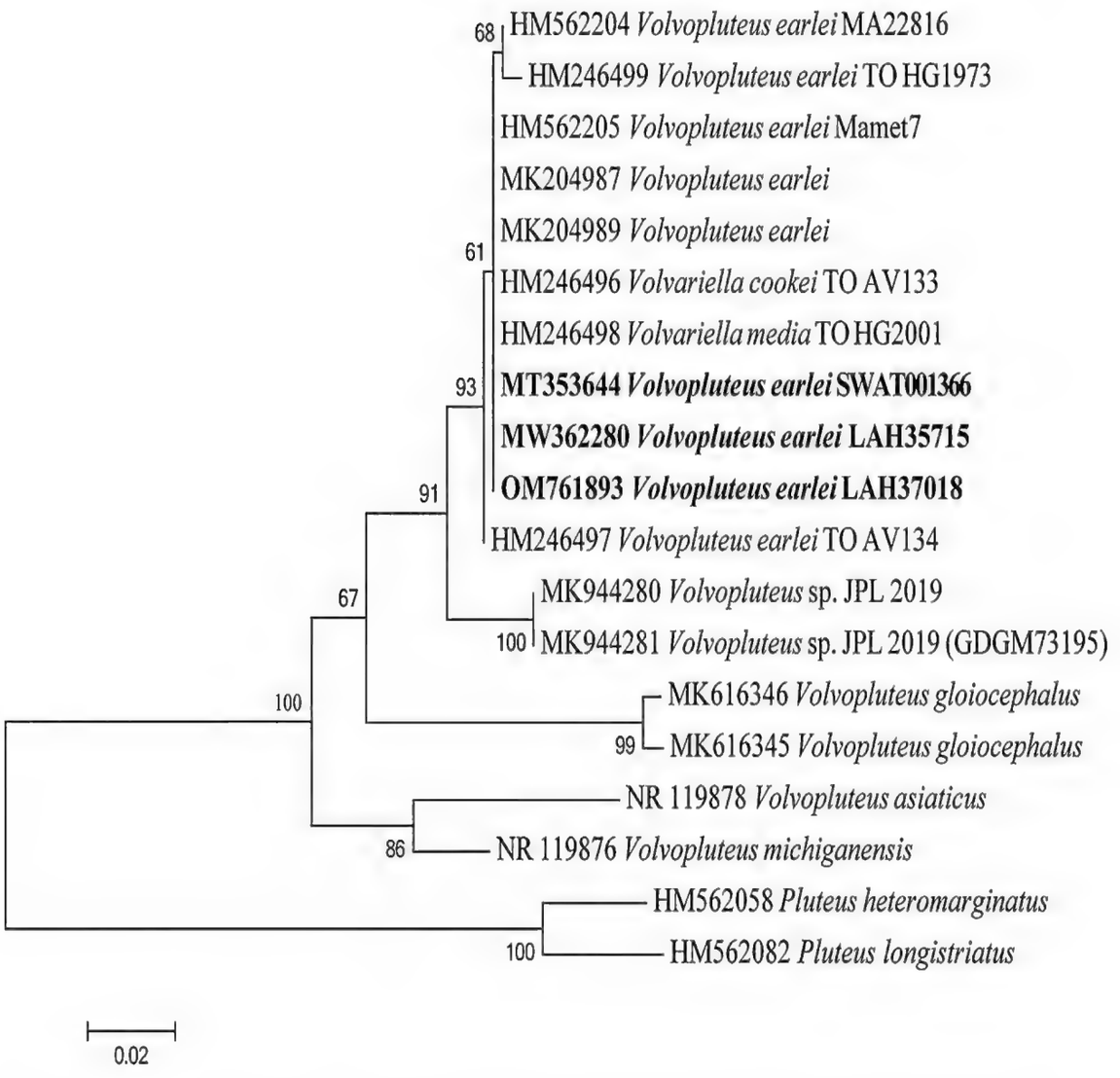

Fig. 2: Maximum likelihood phylogram of *Volvopluteus earlei* and related species inferred from ITS data. Bootstrap values ≥50% are presented above nodes. Newly generated sequences of *V. earlei* are presented in bold.

Taxonomy

Pluteus variabilicolor Babos,
Ann. Hist.-Nat. Mus. Natl. Hung. 70: 93 (1978) Figs 3a–c, 4

Pileus 50–70 mm diam., convex to plano-convex with a central umbo, light orange yellow (10YR 9/8) to dark yellow (2.5Y 9/12), disc darker (2.5Y 7/12); surface smooth, glabrous to lightly velvety, margin striate or not; context concolorous with the pileus just beneath the cuticle, otherwise cream colored, unchanging upon cutting, thin, 2–3 mm at the disc, <1 mm at the margin. Lamellae free, close, sub-ventricose (≤6 mm at the center);

white to pale pink (7.5YR 9/4) when young, pink (7.5R 9/4) when mature; lamellar edge even to slightly undulate; lamellulae present, short to long extending beyond the middle of the lamellae. Stipe 50–80 × 5–7 mm, central, cylindrical to very slightly thickening downward; pinkish (7.5YR 8/4); longitudinally striate in mature specimens, fistulose; context concolorous with the exterior or light yellowish brown (10YR 6/4), unchanging upon cutting.

Basidiospores (5.1–)5.5–6.3(–6.8) × (4.4–)4.8–5.8(–5.9) µm, x = 5.9 × 5.1 µm, Q = 1.1–1.19(–1.2), Q_m = 1.1, mostly sub-globose, rarely ovoid to broadly ellipsoid; smooth, thick-walled (2–3 µm thick), mostly with one central guttula, rarely multi-guttulate. Basidia 20–25 × 6.0–7.0 µm; cylindrical to broadly oblong, with 4 sterigmata. Cheilocystidia 55–90 × 20–30 µm; fusiform with a short appendix at the tip to lageniform. Pleurocystidia 60–120 × 20–40 µm, narrowly utriform to utriform, rare. Pileipellis a hymeniderm composed of clavate to rounded terminal elements, 50–200 × 20–40 µm, without any yellow intracellular pigment. Stipitipellis a cutis composed of narrow branched hyphae, 3.0–5.0 µm diam., with rare claviform caulocystidia, 25–50 × 15–30 µm, usually in clusters extending along the stipe length. Clamp connections absent.

Materials examined: **PAKISTAN, Khyber Pakhtunkhwa, Swat district**, Ingaro Dherai village, 1000 m. a.s.l., on decomposing stump of *Populus nigra* L., 7 August 2014, Junaid Khan ING-32 (SWAT001367; GenBank MT351154); Ingaro Dherai village, 1010 m. a.s.l., on decomposing stump of *Populus nigra*, 27 July 2015, Junaid Khan ING-1507 (LAH31413; GenBank MT351155).

Commentary—*Pluteus variabilicolor*, originally described from Hungary, is characterized by its pileus color varying from yellowish orange to chrome-yellow, pileipellis with morphologically variable elements (a mixture of spheropedunculate-vesiculose and elongated ± cystidioid elements), the presence of caulocystidia, and growth on decaying sawdust (Babos 1978). Our description fits well with previous descriptions (Lohmeyer & al. 1994, Lanconelli & al. 1998, Migliozzi 2011, Lezzi & al. 2014, Kaygusuz & al. 2019).

Lezzi & al. (2014) cite *P. variabilicolor* as widespread but not common. Currently the species has been reported from numerous central and eastern European countries—Hungary (Babos 1978), Austria (Lohmeyer & al. 1994), Italy (Lanconelli & al. 1998, Migliozzi 2011), Germany (Ludwig 2007), central Russia (as *P. castri,* Justo & al. 2011b), Romania (Béres 2012), Slovenia (Jogan & al. 2012), and Moldova (Lezzi & al. 2014). From Asia, the species has been reported from Japan (as *P. castri,* Justo & al. 2011b),

Fig. 3: Field photographs of basidiomata. ***Pluteus variabilicolor***: a–b. (SWAT001367); c. (LAH31413). ***Volvopluteus earlei***: d & f (SWAT001366); e. (LAH37018); g & h. (LAH35715). Scale bars = 10 mm.

and from Turkey and South Korea (Kaygusuz & al. 2019). Here we report *P. variabilicolor* from Pakistan and South Asia for the first time.

Pluteus variabilicolor grows primarily on sawdust deposits and on branches and rotten wood of *Fagaceae*, mainly *Quercus* spp. (Migliozzi 2011, Lezzi & al. 2014). Both Pakistani collections, however, were collected from decaying stumps of *Populus* sp., which agrees with Russian reports of collections growing on *Populus tremula* stumps of (Justo & al. 2011b).

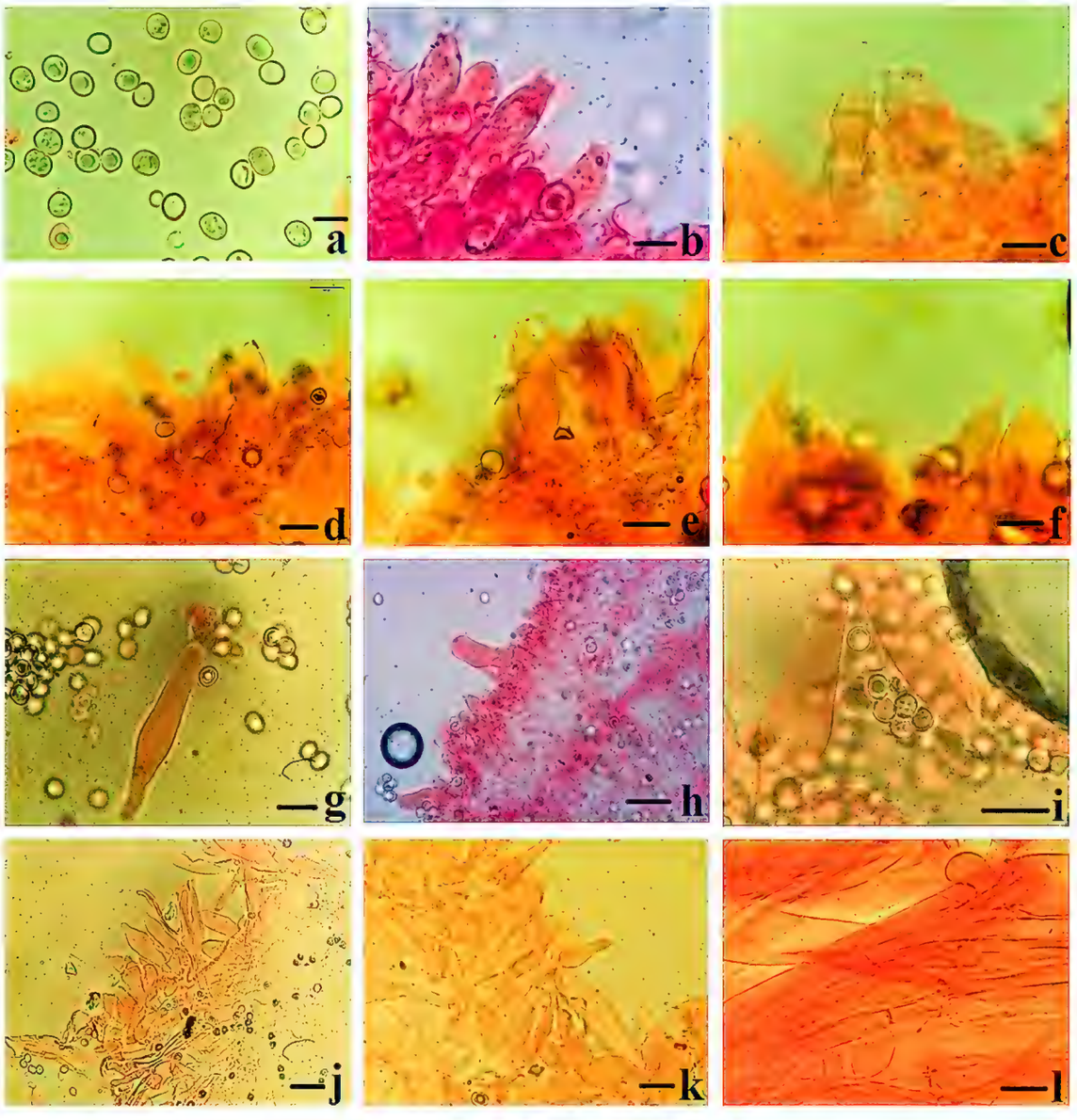

Fig. 4: *Pluteus variabilicolor* (SWAT001367): a. Basidiospores; b. Basidia; c–f. Cheilocystidia; g–i. Pleurocystidia; j, k. Pileipellis elements; l. Stipitipellis elements. Scale bars: a, b = 5 µm; c–i = 20 µm; j–l = 30 µm.

We suggest that *P. variabilicolor* was introduced into Pakistan with the introduction of *Populus nigra.*

Volvopluteus earlei (Murrill) Vizzini, Contu & Justo,
Fungal Biology 115(1): 15 (2011) Figs 3d–h, 5

Pileus 20–40 mm diam.; hemispherical to obtusely conical when young, applanate to plano-concave on maturity; white to greyish white with a greyish disc when young, in maturity white to moderate whitish pink

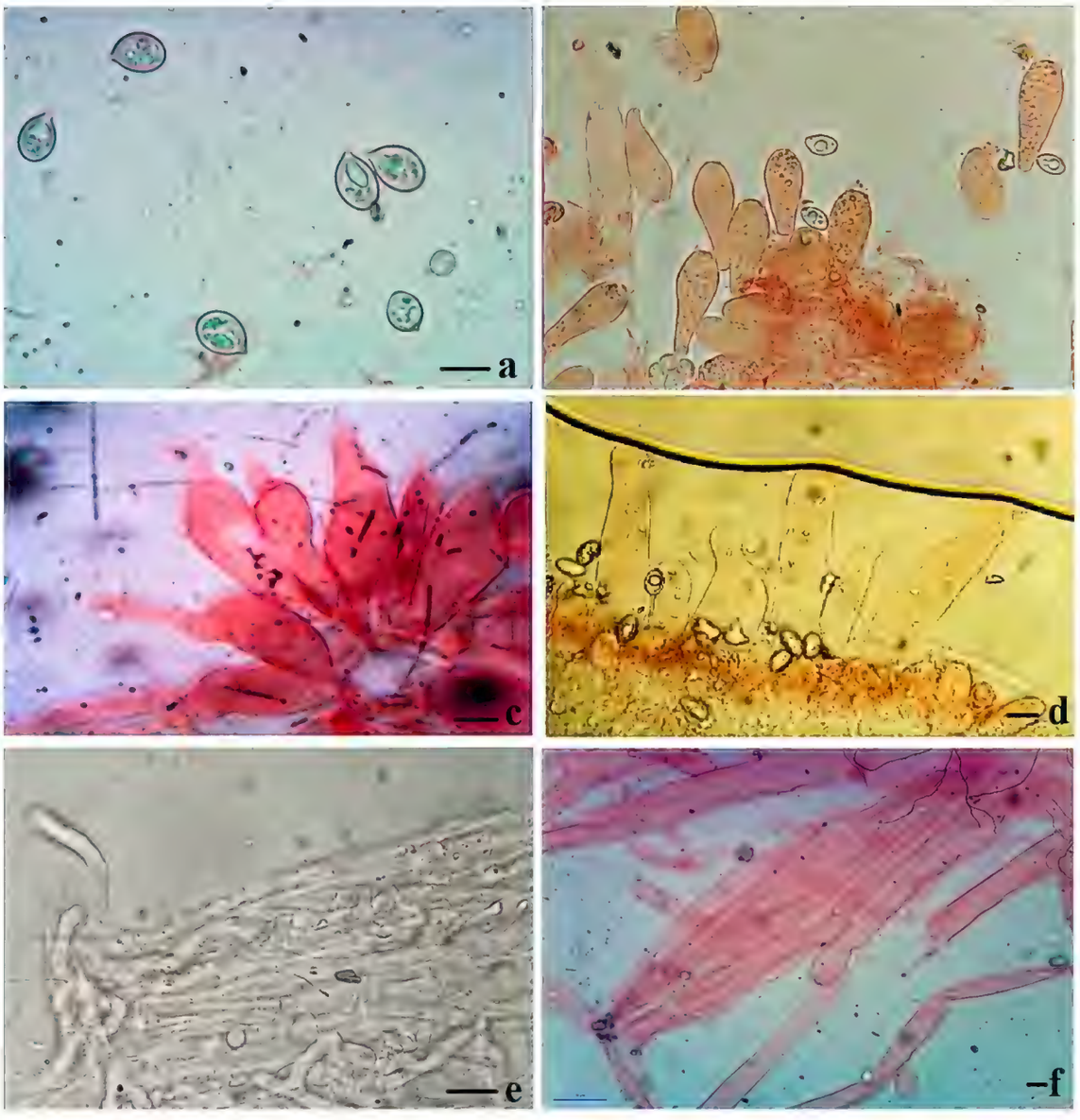

Fig. 5: *Volvopluteus earlei* (LAH35715): a. Basidiospores; b. Basidia; c. Cheilocystidia; d. Pleurocystidia; e. Stipitipellis; f. Pileipellis elements. Scale bars = 10 µm.

(2.5YR 7/4) with reddish pink margins (10R 6/8); surface moist and viscid in young specimens, dry and dull when mature, glabrous; margin striate, striation short (<1/4 of pileus radius); context white, thin, 1–2 mm thick at the disc, ≤1 mm elsewhere. Lamellae free, broad (4–6 mm at the center), sub-crowded to close; white to cream-colored when young, pink (7.5YR 8/8) when mature; lamellulae present. Stipe 70–100 × 4–7 mm; central, tapering upward from slightly flattened base; surface smooth; white to cream colored, turning slightly pinkish (7.5 YR 9/4) upon maturity and handling; volva

present, small (≤8 mm high), membranous, saccate, 2–3 lobed; white to pale gray.

Basidiospores (11.0–)11.5–14.5(–15.0) × (7.0–)7.5–9.6(–10.0) µm, x = 12.8 × 9.1 µm, Q = (1.2–)1.3–1.6(–1.7), Q_m = 1.4; ellipsoid to ovoid, smooth, thick-walled (≤0.5 µm), mostly with one large and many small guttulae. Basidia 30–40 × 10.0–12.0 µm, 4-spored, cylindrical to broadly clavate. Cheilocystidia 35–60 × 8.0–15.0 µm, fusiform to clavate mucronate. Pleurocystidia 80–150 × 15–20 µm, cylindrical to narrowly conical. Pileipellis ixocutis, composed of cylindrical and scattered obovoid to broadly cylindrical and rarely branched terminal elements; 30–35 × 14.0–18.0 µm. Stipitipellis hyphae 7–10 µm diam., ± parallel, caulocystidia cylindrical to narrowly clavate, 40–55 × 8–11 µm. Clamp connections absent.

Material Examined: **PAKISTAN, Khyber Pakhtunkhwa, Swat district**, Ingaro Dherai village, 1000 m. a.s.l., among grasses in humus rich wet soil in the paddy fields, 27 June 2016, Junaid Khan ING-1501 (SWAT001366; GenBank MT353644); **Punjab, Sheikhupura district,** 236 m. a.s.l., solitary on muddy rich soil of a graveyard, 16 July 2017, Aiman Izhar SKP03 (LAH35715; GenBank MW362280); **Muzaffargarh district,** surrounding head Taunsa barrage, 65 m. a.s.l., in scattered groups on humus rich soil, 13 August 2019, Muhammad Haqnawaz TM04 (LAH37018; GenBank OM761893).

Commentary—*Volvopluteus earlei*, originally described from Cuba (Murrill 1911), is characterized by its dry to sub-viscid relatively small (25–45 mm) white pileus, narrowly cylindrical stipe with a small white basal volva, rare or absent broadly clavate pleurocystidia, rostrate cheilocystidia, and large (averaging >12.5 µm long) basidiospores (Shaffer 1957, Desjardin & Hemmes 2001, Contu 2007, Justo & al. 2011a). Murrill (1911) described only basidiospores in his description; other microscopical details were later provided by Coker (1947) and Shaffer (1957). Descriptions from different parts of the world (Pathak 1975, Heinemann 1975, Desjardin & Hemmes 2001, Contu 2007, Vizzini & Contu 2010, Justo & al. 2011a) cite highly diverse cystidial forms for *V. earlei.* For instance, no cystidial forms were reported for the African (Pathak 1975) and Italian (Vizzini & Contu 2010) collections of *V. earlei* [= *Volvariella acystidiata* N.C. Pathak]. Elsewhere, Justo & Castro (2010) reported only cheilocystidia in the Spanish collection, while Shaffer (1957) report both cheilocystidia and pleurocystidia for the North American collections We observed both cheilocystidia and pleurocystidia in the Pakistani collection, which agrees with descriptions of the American collections (Shaffer 1957, Desjardin & Hemmes 2001).

Volvopluteus earlei is known from the U.S.A (Murrill 1911; Coker 1947; Desjardin & Hemmes 2001), Mexico (Vázquez & al. 1989), Africa (Heinemann 1975, as *Volvariella acystidiata*), Sardinia (Contu 2007, as *Volvariella earlei* (Murrill) Shaffer), Spain (Justo & Castro 2010), and India (Amandeep & al. 2015; Atri & al. 1996, as *Volvariella earlei*). This is the first report of this species from Pakistan and the second record from Asia.

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